"Evaluation of Anti-diarrheal Activity of Phytoconstituents isolated from Herbs of Himalayan Region"

A Synopsis

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INTRODUCTION

Diarrhea is the condition of having three or more loose or liquid bowel movements per day. Introduction of the concept is generally attributed to Nobel Prize recipient Eli Metchnikoff, who in 1907 suggested that the dependence in the elderly, particularly those who have been treated with antibiotics for unrelated infections, a toxin produced by *Clostridium difficile* often causes severe diarrhea (Eli Metchnikoffa,1907). The cause is gastroenteritis also. There is little to no structural damage. The loss of fluids through diarrhea can cause dehydration and electrolyte disturbances such as potassium deficiency or other salt imbalances. The most common cause of this type of diarrhea is a cholera toxin that stimulates the secretion, especially anions of chloride ions. *Shigella*, *Entamoeba histolytica*, and *Salmonella* also cause diarrhea. Bacteria generate toxins which can be classified as either exotoxins or endotoxins. Exotoxins are generated and actively secreted endotoxins remain part of the bacteria. Usually, an endotoxin is part of the bacterial outer membrane, and it is not released until the bacterium is killed by the immune system. The lactic acid bacteria (LAB) comprise of Gram-positive, low-GC, acid-tolerant, generally non-sporulating, non-respiring rod or cocci that are associated by their common metabolic and physiological characteristics. These bacteria, usually found in decomposing plants and lactic products, produce lactic acid as the major metabolic end-product of carbohydrate fermentation. Adenovirus types 40 and 41, and viruses cause a significant number of infections. *Campylobacter* spp. are a common cause of bacterial diarrhea, but infections by *Salmonella* spp., *Shigella* spp. and some strains of *Escherichia coli* are also a frequent cause (Rupnik M *et.al*.; 2009). Diarrhea is most commonly due to viral gastroenteritis with rotavirus, which accounts for 40% of cases in children under five. It is a common cause of death in developing countries and the second most common cause of infant deaths worldwide. In 2009 diarrhea was estimated to have caused 1.1 million deaths in people and over and 1.5 million deaths in children. Oral rehydration solutions (ORS) with modest amounts of salts and zinc tablets are the treatment of choice and have been estimated to have saved 50 million children in the past 25 years. In cases where ORS is not available, home-made solutions are often used. Recent discovered Probiotic has also been very beneficial in curing diarrhea. The term Probiotic is currently used to name ingested microorganisms associated with beneficial effects to humans and animals (Doron , *et.al*.; 2008). Antibiotic-associated diarrhea is the best evidence in support of probiotic health

benefits (Arvola, *et.al.;* 1999). Medicinal plants like *Acacia nilotica, Acanthospermun hispidum, Gmelina arborea, Parkia biglobosa and Vitex doniana* used in diarrhoeal treatment. Many ayurvedic herbs such as *Allium cepal* (Piyaz), *Debrege asiasaeneb* (Sandorri), *Justicia adhatoda* (Bhasker), *Pistacia integerima* (Kanger), *Rumex chalepensis* (Hula), *Bergenia ciliate*, *Punica granatum*, *Aegle marmelos (*Bel), *Azadira chtaindica* (Neem), *Fiscus glomerata* (Gular) are used for treatment of diarrhea. The medicinal herbs that will be used for antidiarrheal extract preparation are as follows:

Boswellia serrate: The plant: 3-5 m in length, sometimes longer if grown in a fully stocked forest. Stems: Bark is thin, grayish-green, ashy or reddish with a chlorophyll layer beneath the thin outer layer. Flowers: white, in stout racemes, 10-20 cm long, shorter than the leaves, crowded towards the ends of branches, but not terminal. Fruits: 1.3 cm long, trigonous, with three valves and three heart-shaped. 1-seeded pyrenes, winged, along with the margins. Seeds: solitary in each cell surrounded by a broad membranaceous wing (Kirtikar and Basu, 1987; Nadkarni, 1993). B. serrate is a species characteristic of the tropical dry deciduous forests and occurs in very dry teak forests or in dry mixed deciduous forests. Parts recommended for use as medicine: Bark, Gum Resin.

Phyllanthu samarus: The plant: 10–60 cm tall; main stem simple or branched, terrete smooth younger parts.Stems: 1.5–1.9 mm long, deltoid acuminate blade 1–1.5 mm long. Leaves: 3–11 × 1.5–6 mm elliptic oblong obovate, apiculate at apex, obtuse petioles 0.3–0.5 mm long, stipules 0.8–1.1 mm long triangular Parts recommended for use as medicine: Whole plant.

***Cassia fistula Linn*:** The plant: tree 6-9 m high; trunk straight; bark smooth and pale grey branches spreading, slender. Leaves: Leaves 23-40 cm long; stipules minute, linear-oblong, obtuse, pubescent. Flowers: 30-50 cm. long; pedicels 3.8-5.7 cm. long, slender, pubescent and glabrous.Fruits: 40-70 cm long and 20-27mm in diameter, straight or slightly curved, the striations appearing as fine fissures. Seeds: flat, oval, reddish brown with a well-marked raphe. Parts recommended for use as medicine is whole plant.

Boerhaaviadiffusa Linn: Tree is up to 1 m long or more, having spreading branches. Leaves: Leaves are simple, thick, fleshy, and hairy. The shape of the leaves is up to 5.5 cm to 3.3

cm in area. Flowers: Flowers are minute, subcapitate, present 4–10 together. These are white, pink, or pinkish-red in color. Fruits: ovate, oblong, pubescent, five-ribbed and glandular, anthocarpous, and viscid on the ribs Seeds. Parts recommended for use as medicine areHerb & root.

Butea monosperma: The herb belongs to the plant family Fabaceae and the order Fabales. This herb is commonly known as Palas in Hindi. The parts of the herb that are used for medicinal purposes are its flowers, its leaves the gum obtained from the plant, its seeds and the oil that can be extracted from the flowers as well as the seeds. The flowers of the tree are rich in glucosides, butin, neteroside and butrin. In case of diarrhea flower infusion is given.

Objectives

1. Collection of samples from diarrhea patients.
2. Isolation of microorganisms from diarrheal samples.
3. Identification & maintenance of microorganisms.
4. Extraction of selected medicinal herbal plants using different solvents such as methanol, ethanol and water reflux.
5. To determine ***in vitro*** antidiarrhoeal activities of juices, methanolic extracts and essential oils of ***Boswellia serrate***, ***Phyllanthus amarus, Cassia fistula, Boerhaavia diffusa, Buteamono sperma*** individually and as blends.
6. To measure the minimum inhibitory concentration (MIC) of the selected plant extracts against selected isolates
7. To identify suspected antidiarrhoeal constituents of the active samples and essential oils by GC-MS.

REVIEW OF LITERATURE

Diarrhea is a leading cause of morbidity and mortality globally. As improvements in treatment lead to decreases in diarrhea mortality, it is important to understand the substantial impact of diarrhea morbidity in disability among children and adults worldwide (Fischer-Walker *et.al*.; 2010). Among children diarrhea is responsible for 1.236 million deaths annually and is the second leading cause of death. Data from UNICEF shows that diarrhea is responsible for 14% of in children 1-4 years death in age group 1 and 24% of deaths and is overall responsible for 2.5 million deaths per year (Kiser JD *et.al.;* 2008). Bacterial diarrhea can affect one person or a group of people who all ate the same food by causing bacterial gastrointestitis. It more commonly occurs after eating at picnics, school cafeterias, large social functions, or restaurants. (Wilson M.E *et.al.;* 2005) Over-the-counter medicines such as loperamide (Imodium) and Bismuth subsalicylate (Pepto-Bismol and Kaopectate) may help stop diarrhea in adults. However, people with bloody diarrhea—a sign of bacterial or parasitic infection—should not use these medicines (Clark HF *et.al.;* 1988). If diarrhea is caused by bacteria or parasites, over-the-counter medicines may prolong the problem, so doctors usually prescribe antibiotics instead. These days many research work has been carried out by using the essential oils from the herbal plants for curing diarrhea. An essential oil contains more than 200 chemical components, but some are many times more complex. Essential oils consist of chemical compounds which have hydrogen, carbon and oxygen as their building blocks. They can be essentially classified into two groups: Volatile fraction: Essential oil constituting of 90–95% of the oil in weight, containing the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters. Nonvolatile residue: This comprises 1–10% of the oil, containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoids. However the properties of these components can change. For example, the components from the oils extracted from plants can change according to how, when and where these plants are grown and harvested. The constituents can be again subdivided into 2 groups, such as the hydrocarbons which are made up of mostly terpenes and the oxygena ted compounds which are mainly alcohols, aldehydes, esters, ketones, phenols and oxides. Some of the common components are listed below along with their properties. Alcohols: Alcohols are generally considered safe and have a very low or totally absent toxic reaction in the body or on the skin and so can be used on children. They are extremely useful due to their antiviral, antibacterial and antiseptic properties.

Alcohols are present either as a free compound or combined with a terpene or ester and are found in ylang and lavender as linalool, geraniol in geranium and palmarosa and citronellol found in rose, lemon and eucalyptus. Other alcohols include menthol, nerol and benzyl alcohol. Alderhydes: Alderhydes are found in lemon-scented oils such as Melissa, lemon verbena, citronella etc. and include citral, citronellal and neral. They generally have sedative qualities with specific antiseptic properties. Other known alderhydes include benzaldehyde, cinnamicalderhyde and perillaldehyde. Essential oils containing alderhydes are helpful in treating inflammation, Candida and viral infections.

Moerman, D.E *et.al.;*2014 estimated that there are 250,000 to 500,000 species of plants on Earth. A relatively small percentage (1 to 10%) of these is used as foods by both humans and other animal species. According to his calculations, this leaves approximately 18,000 species of plants which were used for neither food nor drugs. John et.al (2015) have been used for a wide variety of purposes for many thousands of years. These purposes vary from the use of rosewood and cedar wood in perfumery, to flavoring drinks with lime, fennel or juniper berry oil, and the application of lemongrass oil for the preservation of stored food crops. In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapy.

Methodology

- Collection of sample from diarrhea patients from various hospitals in Dehradun.
- Enrichment of 1ml of sample in 10 ml of buffer peptone water aseptically and incubated at 37 ^{0}C for 24 hours.
- Isolation of bacterial species on selective media- Blood agar, XLD, MSA, SM agar, EMB agar, Mac-Conkey agar.
- Identification of microorganisms by following methods:
    - Colony characterization.
    - Gram staining.
    - Biochemical identifications (IMVIC, TSI, Urease Test). (Berge's manual of Bacterial Terminology)
- Identification of antidiarrheal activity of medicinal plants by using Agar well diffusion method. (G.C.Akuodor *et.al.;*2011)
- Quantitative estimation of recovered isolates to check MIC/MBC. MBC test usually uses colony forming units as the measure of bacterial viability and MIC shows the resistance of microorganism to antimicrobial agent by Broth dilution method.(Chang-Geun Kang *et.al.;*2011)
- Isolation of Lactic Acid bacteria and to check antidiarrheal activity of recovered isolates. (Yi-sheng Chen *et.al.;*2010)
- Preparation of antidiarrheal extract by mixing essential oils, herbs and probiotic bacteria. (Ombuna Dinah Nyaitondi, 2013).
- Extraction of oils of different plants and their parts using Clevenger apparatus and check their compatibility with extracted antidiarrheal extract. (D.Pandey *et.al.;*2010).

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